THE DETERMINATION OF ADDITIVE EFFECTS

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(WITH FOUR FIGURES)

It was pointed out in previous papers[1] that in measuring antagonism it is of importance to determine the additive effect; this is the effect produced by dissolved substances in a mixture when each substance acts independently of all the others. It was also stated that when two equally toxic solutions are mixed the additive effect may be predicted, since it will be equal to that of one of the pure solutions. In this discussion it was assumed that if two solutions are equally toxic they will not become unequally toxic when both are diluted to the same degree. This is true (either completely or with negligible error only) for cases which have hitherto come under the writer's observation, but other cases might possibly occur to which it would not apply, and it seems desirable to discuss briefly the treatment of such cases.

As an example of this we may consider the influence of dilution on the effects of two solutions, *A* and *B*. These may be mixtures, but for the sake of simplicity we may assume that they are pure solutions of two salts, *A* and *B*, and that 100 cc. of solution *A*, or of solution *B*, diluted to 200 cc. will permit the same amount of growth to take place, as shown in fig. 1. In this figure the abscissas represent growth, while the ordinates represent the number of cc. which are taken and diluted to make 200 cc. of the culture solution. Thus on the curve *A*, *A*, the abscissa at 60 represents the growth in a culture solution made by taking 60 cc. of solution *A* and adding water to make 200 cc. Similarly on the curve *B*, *B*, the abscissa at 40 represents the growth in a culture solution made by taking 40 cc. of solution *B* and adding water to make 200 cc.

Ordinarily we should expect these curves to be almost or quite identical, but we may imagine cases in which they diverge, as shown in fig. 1. It is apparent from the figure that while 100 cc. of either

[1] BOT. GAZ. 58:178, 272. 1914.

solution (diluted to 200 cc.) produces exactly the same effect, 50 cc. of solution *A* (diluted to 200 cc.) produces a different effect from 50 cc. of *B* (diluted to 200 cc.).

Let us now consider an antagonism curve obtained by growing plants in a culture solution made by mixing the two solutions, *A* and *B*, so as to make 100 cc. of mixture, which is then diluted to 200 cc.

The result of growing plants in such mixtures may be expressed by a curve, as shown in fig. 2. In this figure the ordinates represent growth, while the abscissas represent the number of cc. of solution *A*, or of solution *B*, taken (and diluted to 200 cc.) to make up the culture solution. Thus, *A* 60, *B* 40 means that 60 cc. of solution *A* was mixed with 40 cc. of solution *B* and sufficient water added to make 200 cc.

To measure the amount of antagonism at any point on this curve according to the method outlined in previous papers,[2] we must first determine the additive effect. To ascertain this at any point,

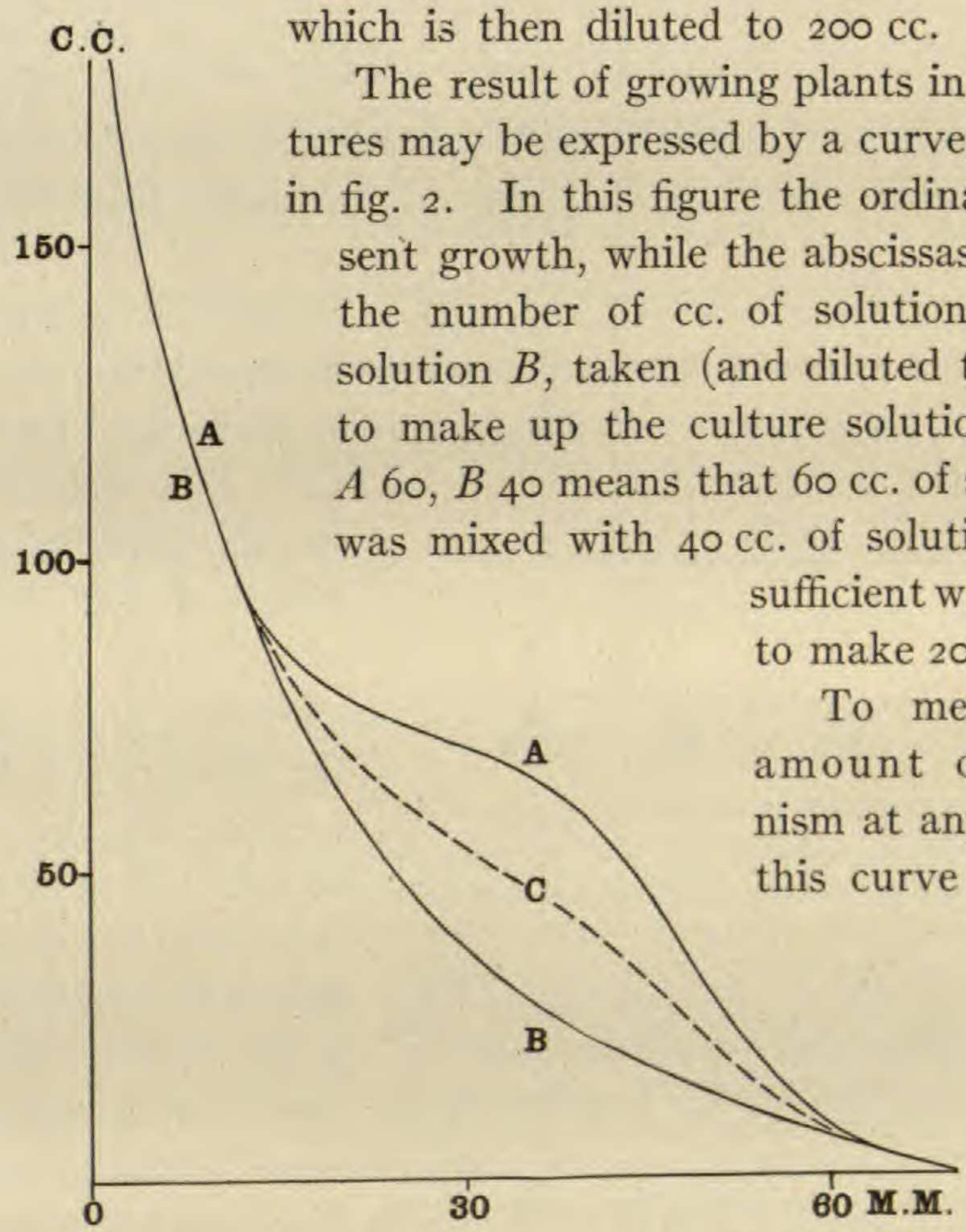

FIG. 1.—Curves showing growth in various dilutions of two solutions of salts, *A* and *B:* the abscissas represent growth; the ordinates represent the number of cc. of the salt solution which are mixed with water to form 200 cc. of the culture solution in which the plants were grown; the two salt solutions are equally toxic at certain concentrations but not at others; the curve *C* is drawn by taking points half-way between *A* and *B* (measured vertically); it serves as a basis of comparison in computing additive effects.

[2] BOT. GAZ. 58:178, 272. 1914.

as for example at *A* 60, *B* 40, in fig. 2, we must answer the question: What is the effect of 60 cc. of *A* + 40 cc. of *B* + 100 cc. of water, when each salt acts independently of the other? It is obvious that we cannot answer this by merely adding together the abscissas at these points on the curves[3] in fig. 1. Since this cannot be done, it might be thought feasible to express the effect of *A* in terms of *B*, or vice versa. If the curves *A*, *A*, and *B*, *B*, in fig. 1 were identical, this would be very simple, since the additive effect of (60 *A* + 40 *B*) would equal the additive effect of (60 *A* + 40 *A*) or the effect of 100 *A*, which is shown by the curve to be 11. Proceeding in this way, we should find the additive effect at any point on the antagonism curve to be exactly the same (that is, 11), and the additive effect could therefore be represented by a straight horizontal line, as is done in fig. 2 (dotted line).

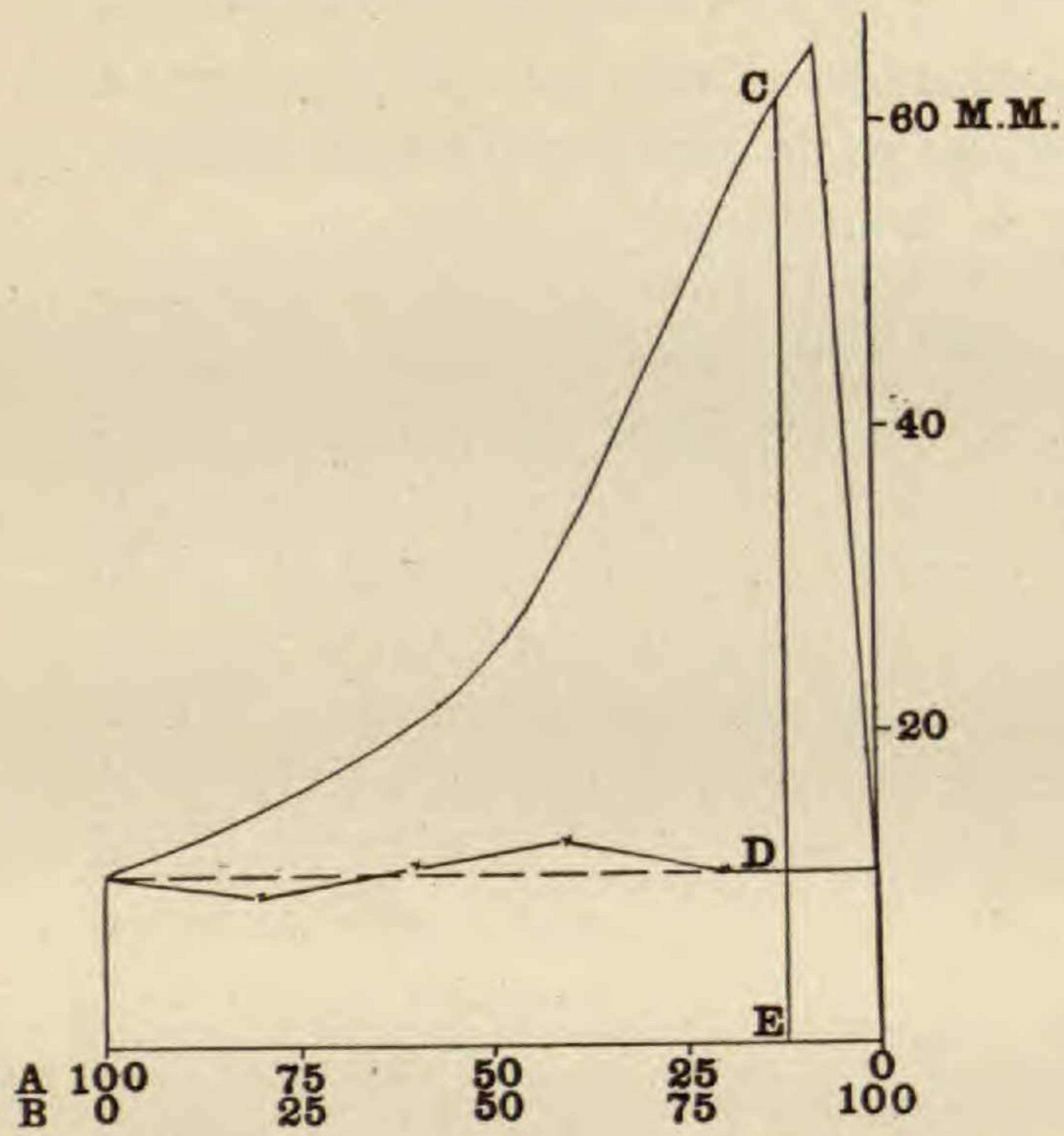

FIG. 2.—Antagonism curve showing growth in various mixtures of solutions of the two salts, *A* and *B*, the dilution curves of which are shown in fig. 1: the ordinates represent growth; the abscissas represent the number of cc. of the salt solutions which are mixed with water to make 200 cc. of the culture solution in which the plants were grown; thus *A* 75, *B* 25 signifies that 75 cc. of solution *A* were added to 25 cc. of solution *B*, the whole being then diluted to form 200 cc.; the additive effect (calculated by the method explained in the text) is shown as an unbroken line; the additive effect which would be obtained if the two dilution curves in fig. 1 did not diverge is shown as a horizontal dotted line; the antagonism at the point *C* is *CD* ÷ *DE*.

But when the curves diverge, as in fig. 1, we cannot consider the effect of 40 *B* as equal to that of 40 *A*; we see by inspection

[3] This is evident, for example, from the fact that the abscissa at 50 on curve *AA* is not equal to exactly twice the abscissa at 100 on curve *AA*; the abscissa at 30 is not equal to exactly twice the abscissa at 60, etc.

of the figure that the growth in 40 *A* is 47, while that in 40 *B* is 28.5 (this is equivalent to the growth in 71 *A*). The additive effect of (60 *A*+40 *B*) is therefore equal to the effect of (60+71=) 131 *A*, which gives (as read from the curve in fig. 1) the additive effect 6.

If we calculate the additive effect of the same mixture in terms of *B*, we find that the effect of 60 *A* is 39, which is equal to the effect of 24 *B*. Hence the additive effect of (40 *B*+60 *A*) equals the effect of (40 *B*+24 *B*=) 64 *B*, which gives as the additive effect 19.5.

TABLE I

ADDITIVE EFFECT WHEN THE EFFECT OF *M* = THE EFFECT OF 2 *N*

Mixture which is diluted to 200 cc. to make the culture solution	Additive effect
100 cc. *M*	1.0
80 " *M*, 20 " *N* = 90 *M*	2.0
60 " *M*, 40 " *N* = 80 *M*	3.3
40 " *M*, 60 " *N* = 70 *M*	5.0
20 " *M*, 80 " *N* = 60 *M*	7.5
100 " *N* = 50 *M*	11.0

We have in this case, therefore, two values for the additive effect, namely 6 and 19.5. One is undoubtedly too high, the other too low. Instead of taking the mean (or the weighted mean) of these two values, it seems desirable to avoid this complication altogether by calculating *A* and *B* in terms of a third curve, *C*. This may be obtained by taking points midway between the two curves *A* and *B* (the distance being measured vertically) and drawing a line through them, giving the dotted line *C*. The curve *C* could be drawn in any convenient manner (it might, for example, be a parabola or a hyperbola), but it should have two points in common with each of the other curves. This might be arranged by multiplying or dividing the ordinates or abscissas so as to make these curves coincide at the origin and at the half-way point with the arbitrary standard curve.

By this method we find that the effect of 60 *A* is equal to the effect of 42 *C*, while the effect of 40 *B* is equal to the effect of 56 *C*. The additive effect of (60 *A*+40 *B*) is therefore equal to the additive effect of (42 *C*+56 *C*=) 98 *C*, which is seen from the figure to be 11.5.

The values of the additive effect thus obtained are plotted in fig. 2. It will be seen that these values do not differ greatly from the value of an additive effect which is constant (and equal to the effect of 100 *A* or 100 *B*). Unless, therefore, the two dilution curves (as plotted in fig. 1) diverge widely, there will be no great error in regarding the additive effect as constant (and equal to the effect of 100 *A* or 100 *B*); this error will in fact ordinarily be less than the experimental error.

In case there are several salt solutions to be mixed, we may draw the corresponding dilution curves and average the ordinates of these curves at various elevations to obtain points through which a curve may be drawn which shall serve the same purpose as the curve *C* in fig. 1; or an arbitrary curve (for example, a parabola or hyperbola) may be drawn for this purpose.

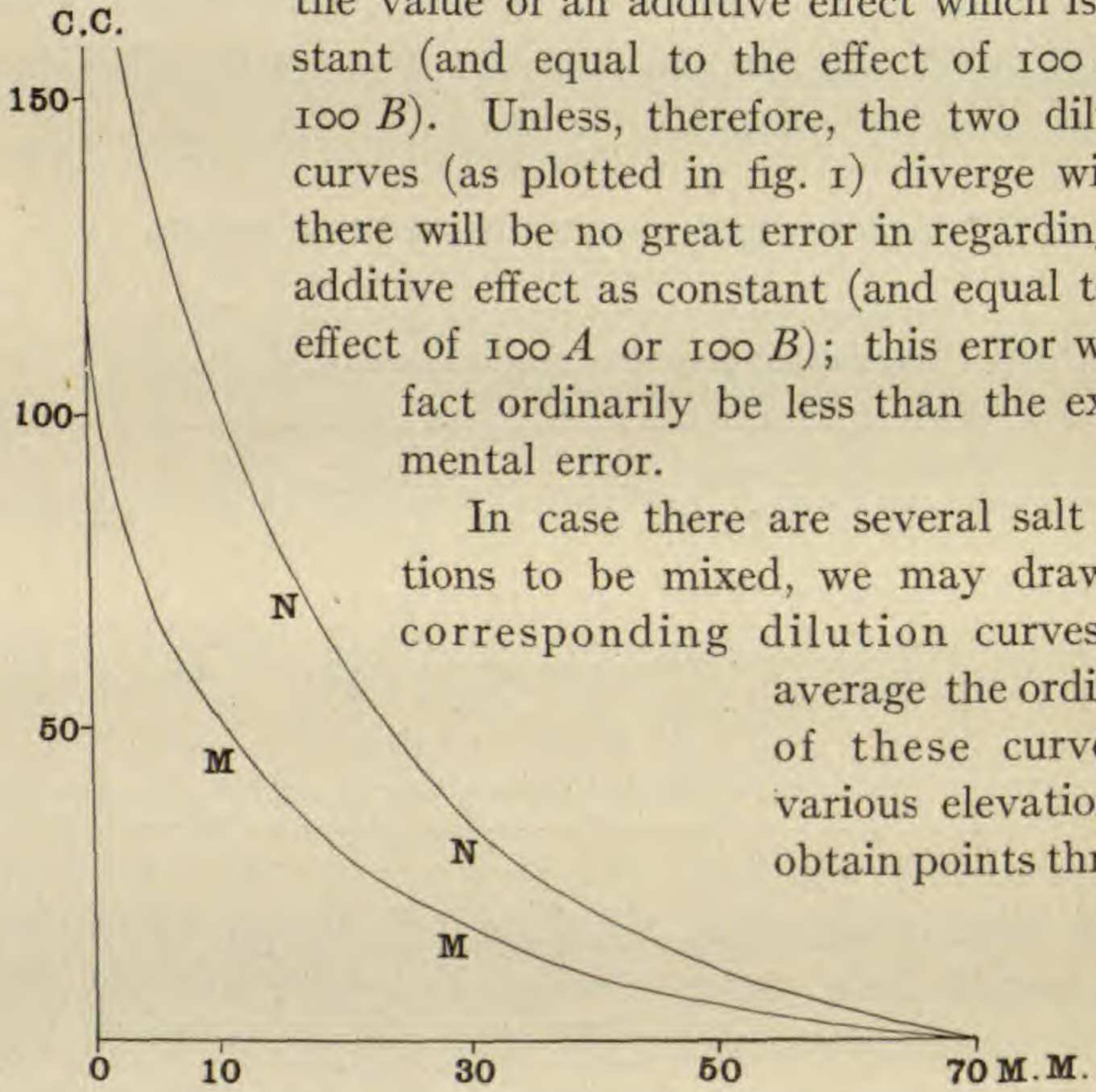

FIG. 3.—Curves showing growth in various dilutions of two unequally toxic solutions of salts, *M* and *N*: the abscissas represent growth; the ordinates represent the number of cc. of the salt solution which are taken and diluted to 200 cc. to make the culture solution in which the plants are grown.

When the two salt solutions are not equally toxic, we often find cases in which a constant relation exists between the amounts of the two solutions which (diluted to 200 cc.) produce the same effect. For example, it will be seen in fig. 3 that a growth of 20 mm. may be found at 30 *M* or 60 *N*; or a growth of 15 mm. may be found at 40 *M* or 80 *N*. If this relation holds throughout (even approximately), we may consider the effect of *M* as equal to the effect of 2 *N*. If we grow plants in culture solutions made by taking sufficient of $M+N$ to make 100 cc. (and then diluting this mixture to 200 cc.), we shall get an additive effect which is not constant but which will always be the same for any given mixture, whether calculated as *M* or as *N*. The additive effect obtained under these conditions is shown as a curved and dotted line in fig. 4 (cf. table I). This procedure, as will readily be seen, is the same as determining the additive effect of *M* mixed with another solution of *M* which has been diluted to a definite degree with water (each 100 cc. of the mixture of $M+[M$ diluted] being itself diluted to 200 cc.). This is in fact the method suggested in a previous paper.[4]

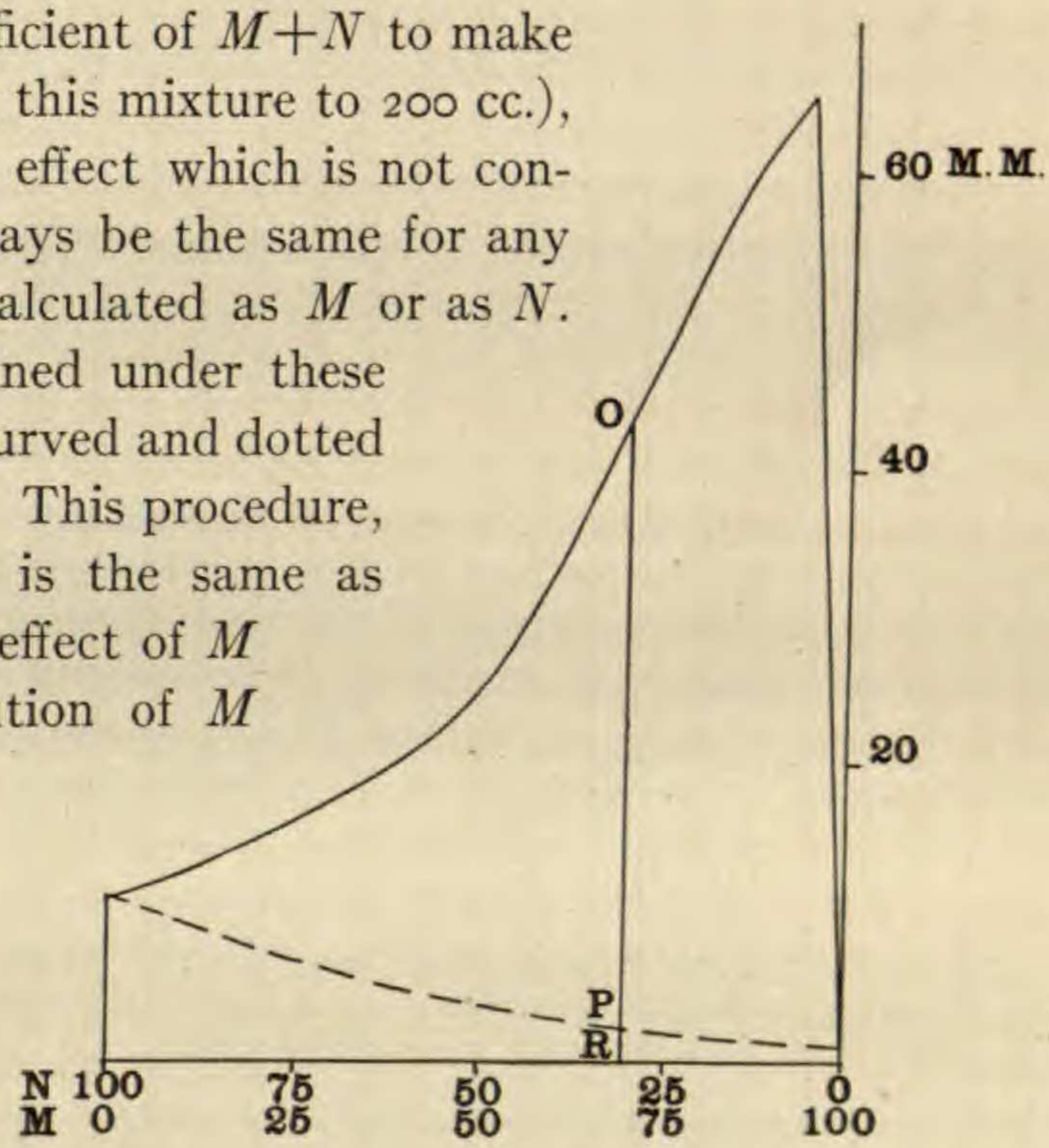

FIG. 4.—Antagonism curve showing growth in various mixtures of two solutions of salts, *M* and *N*, of which the dilution curves are shown in fig. 3: the ordinates represent growth; the abscissas represent the number of cc. of the solutions of salts which are taken and diluted to 200 cc. to make the culture solution in which the plants were grown; thus *N* 75, *M* 25 signifies that 75 cc. of *N* were added to 25 cc. of *M* and the whole diluted to 200 cc.; the additive effect is shown by the curved dotted line; the antagonism at the point *O* is $OP \div PR$.

A relation such that the effect of $M =$ the effect of $\times N$ will be found to hold (at least approximately) in most cases. If it does not, the additive effect may be calculated in terms of a third curve drawn arbitrarily or by taking points midway between the two (measured vertically), as previously explained.

[4] BOT. GAZ. 58:178. 1914.

When the solution contains more than two components, we may follow a method similar to that already outlined for equally toxic solutions containing more than two components.

In most cases the calculations described above may be dispensed with, as the error (if any) in proceeding by the method described in the two preceding papers[5] is so small as to be negligible. Calculations such as are here discussed will be necessary only in those cases in which equally toxic solutions acquire, when diluted to the same degree, a very marked difference in toxicity, or in cases where a mixture is made of unequally toxic solutions which have dilution curves of very dissimilar character.

Summary

In most cases two solutions which are equally toxic remain so (at least approximately) when both are diluted to the same degree; this allows the additive effect to be easily determined. But in exceptional cases, where this does not hold, a value may be assigned to the additive effect.

Similar considerations apply to unequally toxic solutions.

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[5] *Op. cit.*